Wayamba Journal of Animal Science – ISSN: 2012-578X; P1020-P1027, 2014
First Submitted July 14, 2014; Number 1405325458

INDUCTION OF SYNERGISTIC POTENCY OF VITAMIN A AND SPIRULINA AGAINST PERSISTENT ARSENIC POISONING IN MICE

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A study was conducted to evaluate the induction of potency of Spirulina combined with vitamin A on persistent arsenicosis on hemato-biochemical alteration in white mice. Sixty white mice were randomly divided into 5 groups viz: control group (T0), Arsenic (As) treated group (T1), Arsenic + Spirulina treated group (T2), Arsenic + Vitamin A treated group (T3) and Arsenic + Spirulina + Vitamin A treated group (T4). Mice in Group T0 were given feed ad-libitum and water. Mice in Groups T1, T2, T3 and T4 were treated orally with 4 mg of sodium arsenite/kg body weight daily, for 63 days. In addition to the sodium arsenite, the mice in groups T2 and T4 were simultaneously fed with spirulina 1 mg/kg of feed ad-libitum while groups T3 and T4 were fed vitamin A at 2500 IU/kg of feed ad-libitum up to 63 days, respectively. The values of Serum Glutamate Oxaloacetate Transaminase (SGOT) enhance significantly (P<0.01) in all the treated groups of mice (T1, T2, T3 and T4) compared to the control (T0) group, but Spirulina combined with Vitamin A produced values significantly comparable to the untreated control group. Whereas Serum Glutamate Pyruvate Transaminase (SGPT) showed minor significance differences among the treatment groups, Spirulina combined with Vitamin A appeared most effective in managing arsenic treatment. Spirulina + Vitamin A increased the values of Total erythrocyte count (TEC), Total leukocyte count (TLC) and Hemoglobin (Hb) against arsenic toxicity in mice but showed no significance differences. The combination of Spirulina and vitamin A were found more successful in the prevention of Persistent arsenicosis in mice than using these substances (Spirulina or Vitamin A) only

Key words: Persistent arsenicosis, Spirulina, Vitamin A, hemato-biochemical alteration, Mice.

Arsenic, a metalloid, occurs naturally, being the twentieth most abundant element in earth's crust and is a component of more than 245 minerals. The inorganic forms consisting mostly of arsenite and arsenate compounds are toxic to human health. Arsenic-related diseases remain a problem of serious public health concerns in many countries including Argentina, Bangladesh, Chile, China, India, Mexico, Thailand and the USA (Smith et al., 2000; Khalequzzaman et al., 2005; WHO, 2011). Chronic arsenic poisoning results from drinking contaminated well water over a long period of time and the World Health Organization recommends a limit of 0.01 mg/L (10 ppb) of arsenic in drinking water, though this limit also can predispose to arsenicosis (WHO, 2001; Walker and Fosbury, 2009; Prozialeck et al., 2008). More recent findings show that consumption of water with levels as low as 0.00017 mg/L (0.17 ppb) over long period of time can still lead to arsenicosis (WHO, 2001). W hen used alone or in combination with vitamins and/or minerals was found to be effective in the removal of arsenic from arsenic-loaded tissues in various species including man (Misbahuddin et al., 2006; Awal, 2007). It is also used as oral supplemen- tation with vitamin A (retinol) in the treatment of cutaneous arsenicosis as recorded by Ahmad et al. (1998).

Arsenicosis presents with significant changes in the Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), serum creatinine, urea, uric acid levels and various hemato- logical parameters like TEC, TLC, Hb, blood

sugar level in the Swiss albino rats (Yasmin et al., 2011). Acute, intermediate or chronic exposure to arsenic similarly resulted in the development of anemia and leucopenia in a previous study (Flora et al., 2007). This study was undertaken to determine the effect(s) of Spirulina and vitamin A, including their combination on some hemato- logical and biochemical parameters in White mice with experimentally-induced chronic arsenicosis.

MATERIALS AND METHODS

Procedure of sodium arsenite solution preparation

On the basis of the mean total body weight of the rats, a daily dose of 4 mg/kg bodyweight of sodium arsenite ($NaAsO_2$) was weighed out and administered to each group via the drinking water. To ensure that the daily dosage was taken, 10 ml drinking water per group was initially allotted for mixing $NaAsO_2$ and only after the drinker is completely empty was fresh water served ad libitum.

Procedure of preparation of Spirulina powder preparation

Each tablet of Spirulina (containing 500 mg of *Spirulina platensis*) was made to a homogeneous powder with the help of pestle and mortar. Then the required amount of Spirulina was measured with the help of electric balance. The powdered Spirulina was kept in desiccators to prevent water absorption and change in quality of the product. For proper homogeneity, small amount (≈15 ml) of distill water was added to make a suspension and then the suspension was added drop by drop to the feed with the help of a small stainless steel spoon and simultaneously the feed was stirred by a glass rod for homogenous mixing. After completion of the mixing, the mixed feed was dried in an electric oven at 50ºC for 24 h and kept in air-tight plastic container.

Procedure of vitamin A preparation

Each vitamin A capsule contained 50,000 I.U of vitamin A (Ovit-A Capsule, 2500 IU/kg feed) was mixed with 2 kg dried pellet feed. For proper mixing small amount (≈10-15 ml) of distill water was added to make an emulsion and the emulsion was added drop by drop to the feed with the help of a small stainless steel spoon and simultaneously the feed was stirred by a glass rod for homogenous mixing. After completion of the mixing, the mixed feed was dried and kept in air-tight plastic container.

Groups and feed

Sixty (60) white mice were purchased for use in this study. They were conditioned to the new environment and given feed and water ad-libitum prior to treatment. Each rat was weighed and the sixty animals were randomly divided into 5 groups of 12 rats each viz: control group (T0), Arsenic (As) treated group (T1), Arsenic + Spirulina treated group (T2), Arsenic + Vitamin A treated group (T3) and Arsenic + Spirulina + Vitamin A treated group (T4). Mouse in Group T0 was given feed ad-libitum and water. Mouse in Groups T1, T2, T3 and T4 were treated orally with 4 mg of sodium arsenite/kg body weight daily, for 63 days ($NaAs_2O_2$, MW 197.84 g/mol, May & Baker Ltd, Dagenham, England). In addition to the sodium arsenite treatment, the mice in groups T2 and T4 were simultaneously fed with Spirulina (Life Line International Company, Bangladesh) at 1 g/kg of feed *ad-libitum* while groups T3 and T4 were fed vitamin A (OVIT-A®; Opsonin Pharma Limited; Bangladesh) at 2500 IU/kg of feed ad-libitum up to 63 days respectively. All animals were fed ad libitum throughout the period of the experiment.

Procedure of sampling

Starting from day 21 post-treatment, and every 21 days thereafter, 4 mice from each group were anesthetized using chloroform and approximately five milliliters (ml) of blood was collected directly from heart of each mouse by using sterile syringe. The blood from each mouse was then transferred into three tubes each. For the biochemical test, blood was taken into pre-marked centrifuge glass test tubes immediately after collection and was kept at room temperature for 1 hr without agitation to

clot with a view to collect serum. The harvested sera were kept at-20^{O}C until used. For the hematological parameter test and arsenic determination in blood, 1 ml of each blood was taken separately into 2 EDTA coated tubes. Bloods samples for hematological investigation were preserved at +4^{O}C and those for arsenic level in blood at -20^{O}C until tested. All blood samples were taken on day 21, day 42 and day 63.

Biochemical tests

Sera were thawed on the laboratory bench (≈+25^{O}C) and the SGOT, SGPT were determined through the use of Reflotron® Plus (Boehringer Mannheim, Germany) according to the method described by Deneke and Rittersdorf (1984) and Deneke et al. (1985).

Determination of hematological parameters

Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC) and Hemoglobin concentration (Hb) were determined using the method described by Lamberg and Rothstein (1977).

Statistical analysis

Since the experimental data were designed following the complete randomized design (CRD), it was statistically analyzed using one- way analysis of variance (ANOVA) and SPSS v13 software. Mean comparisons of the treatments were made by the Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1980).

RESULTS

Biochemical parameters:

Serum Glutamate Oxaloacetate Transaminase (SGOT) activity

The values of SGOT on day 21 ranged between 230.58 to 321.33 U/L and these values increased to 234.67 to 380.51 U/L and 231.00 to 401.09 U/L by days 42 and 63 respectively (Table 1). The highest values were observed in the arsenic only group (T_1) while the lowest values were observed in the control group. There were significant differences within the groups during the three days (21, 42 and 63) of measurement ($P<0.01$). It appears that while Spirulina alone has some effect in lowering the SGOT values in response to prolonged administration of arsenic, the combination of Spirulina and Vitamin A produced a more significant reduction in SGOT level comparable to the control group ($P<0.01$, Table 1). Vitamin A administration alone did not significantly reduce the effect of arsenicosis on SGOT level. Overall, chronic arsenicosis significantly increased the level of SGOT in the blood by up to 39.35% (90.75 U/L) to 73.63% (401.09U/L; Table 1).

Serum Glutamate Pyruvate Transaminase (SGPT) activity

Continuous administration of arsenic to white mice caused a significant increase in the blood SGPT level to between 15.90% (6.86 U/L) and 39.21% (16.67U/L). There was a huge surge in values of SGPT on day 21 in response to arsenic administration but these values reduced slightly over the next 42 days (Table 1). The highest values of SGPT were observed in the arsenic only group (T_1) while the lowest values were observed in the control group. There were significant differences within the groups during days 21 and 42 but this difference became insignificant by day 63 (Table 1). It is probable that prolonged administration of arsenic will be followed in a long term by apparently normal levels of SGPT ($P<0.01$). Though both Spirulina and vitamin A (individually or in combination) redu 9 level of SGPT in response to administration, there was no significant difference between the treatments. On day 42, the values of SGPT was the highest in T_1 group mice and lowest in control group and the As values were significantly ($P<0.01$) increased in T_1, T_2 and T_3 group mice compared to control group mice. But the As values of T_4 group mice were not significant compared to control group mice. The values in T_2 and T_3 were statistically significantly different ($P<0.01$) compared to T_1 and T_4 group mice and the difference in As content between T_2 and T_3 group rats were not significant. However the As contents increased in T_2

Table 1. Potency of different treatment on biochemical parameters (SGOT, SGPT values) in mice.

Biochemical parameter			
SGOT(U/L)			
Treatment	**Day 21**	**Day 42**	**Day 63**
Control(T_0)	230.58 ± 0.88^{c}	234.67 ± 2.40^{c}	231.03 ± 3.79^{e}
Arsenic (T_1)	321.33 ± 2.03^{a}	380.51 ± 2.92^{a}	401.09 ± 0.1^{a}
Spirulina + Arsenic(T_2)	294.33 ± 6.31^{b}	281.05 ± 1.73^{b}	282.09 ± 5.57^{c}
Arsenic+ Vit A (T_3)	314.00 ± 5.86^{a}	312.61 ± 2.33^{a}	306.121 ± 5.54^{b}
Spirulina + Arsenic + Vit A(T_4)	240.00 ± 1.28^{c}	240.33 ± 1.81^{c}	247.18 ± 2.03^{d}
Level of significance	P<0.01	P<0.01	P<0.01
SGPT(U/L)			
Treatment	Day 21	Day 42	Day 63
Control(T_0)	42.51 ± 1.17^{c}	42.91 ± 1.41^{c}	43.12±1.10
Arsenic (T_1)	59.18 ± 2.10^{a}	50.73 ± 1.03^{a}	49.98±1.80
Spirulina + Arsenic(T_2)	45.12 ± 1.15^{b}	46.67 ± 1.41^{b}	44.41±2.18
Arsenic+ Vit A (T_3)	47.49 ± 1.05^{b}	47.81 ± 0.07^{b}	44.75±1.07
Spirulina + Arsenic + Vit A(T_4)	45.28 ± 1.52^{b}	42.70 ± 1.09^{c}	43.63±1.13
Level of significance	P<0.01	P<0.01	NS

Values indicate the mean±SE; NS = no significant difference between values; Values with similar superscripts did not differ significantly; Values with dissimilar superscripts differed significantly. Evaluation was done using Duncan multiple range test (DMRT).

and T_3 group but decreased in T_1 and T_4 groups on day 42 compared to day 21. On day 63 the SGPT values were highest in T_1 and the lowest in T_0 group mice but the difference were statistically not significant among themselves. However the As contents increased in T_4 group but decreased in T_1, T_2 and T_3 groups on day 63 compared to day 42 (Table 1).

Hematological parameters

Total erythrocyte count (TEC)

Total erythrocyte counts on day 21 was found highest in T_0 group mice and lowest in T_1 group mice but the differences were not statistically significant among all groups of mice.

On day 42, total erythrocyte count was found highest in T_0 and lowest in T_1 group of mice. The differences were found significant (P<0.05). The As values of T_2, T_3 and T_4 group mice were statistically non significant compared to control group but the As values of T_1 group Mouse were statistically significant compared to control group. The differences of As value among T_1, T_2, T_3 and T_4 group Mouse were statistically non significant. However the As con- tents increased in T_2, T_3 and T_4 group but decreased in T_1 groups on day 42 compared to day 21. Total erythrocyte counts on day 63 was found highest in control group mice and lowest in T_1 group mice but the difference were not statistically significant among all group of mice. However the As contents increased in all treated group on day 63 compared to day 42 (Table 2).

Total leukocyte count (TLC)

Total leukocyte counts on day 21 was found highest in control group mice and lowest in T_1 group mice but the difference were not statistically significant among all group of mice . But the values gradually increased by 42 days compared to day 21 value. On day 42, total leukocyte counts was found highest in control group mice and lowest in T_1 group mice but the differences were not statistically significant among all group of mice. However the As contents were increased in all treated groups on day 42 compared to day 21. Total leukocyte counts on day 63 was found highest in control group mice and lowest in T_1 group mice but the differences were not

Table 2. Potency of different treatment on hematological parameters (Total Erythrocyte Counts, Total Leukocyte Counts and Hemoglobin concentration) in mice.

Hematological parameter			
Total Erythrocyte Count (TEC) million/ µl			
Treatment	**Day 21**	**Day 42**	**Day 63**
Control(T_0)	7.15 ±0.35	8.61±0.21[a]	8.02±0.71
Arsenic (T_1)	6.42±0.92	5.87±0.19[b]	7.11± 0.20
Spirulina + Arsenic(T_2)	7.09±0.41	7.21±0.51[ab]	7.48±0.25
Arsenic+ Vit A (T_3)	6.36±0.21	7.81±0.26[ab]	7.85±0.31
Spirulina + Arsenic + Vit A(T_4)	7.67±0.29	7.93±0.31[ab]	8.92±0.28
Level of significance	NS	P<0.05	NS
Total Leukocyte Count (TLC) thousand/ µl			
Treatment	**Day 21**	**Day 42**	**Day 63**
Control(T_0)	9.13±0.28	10.81±0.51	10.78±0.42
Arsenic (T_1)	9.02±0.12	10.27±0.27	9.71±0.58
±Spirulina + Arsenic(T_2)	9.11±0.38	10.35±0.54	9.78±0.64
Arsenic+ Vit A (T_3)	9.08±0.39	10.29±0.21	9.77±0.62
Spirulina + Arsenic + Vit A(T_4)	9.12±0.71	10.72±0.82	10.68±0.68
Level of significance	NS	NS	NS
Hemoglobin Concentration(Hb) gm/dl			
Treatment	**Day 21**	**Day 42**	**Day 63**
Control(T_0)	10.71±0.62	11.32±0.51	11.87±0.12
Arsenic (T_1)	9.92±0.31	9.42±0.44	10.22±0.41
±Spirulina + Arsenic(T_2)	10.19±0.32	10.54±0.71	11.28±0.51
Arsenic+ Vit A (T_3)	10.01±0.31	10.52±0.76	10.62±0.22
Spirulina + Arsenic + Vit A(T_4)	10.51±0.34	10.78±0.72	11.81±0.71
Level of significance	NS	NS	NS

Values indicate the mean±SE; NS = no significant difference between values; Values with similar superscripts did not differ significantly; Values with dissimilar superscripts differed significantly. Evaluation was done using Duncan multiple range test (DMRT).

statistically significant among all group of mice. However the As contents decreased in all treated groups on day 63 compared to day 42 (Table 2).

Hemoglobin concentration (Hb)

Total Hemoglobin concentration (Hb) on day 21 was found highest in control group mice and lowest in T_1 group mice but the differences were not statistically significant among all groups of mice. On day 42, total hemoglobin concentration (Hb) was found highest in control group mice and lowest in T_1 group mice but the differences were not statistically significant among all groups of mice.

However the As contents increased in T_2, T_3 and T_4 groups but decreased in T_1 groups on day 42 compared to day 21. Total Hemoglobin concentration (Hb) on day 63 was found highest in control group mice and lowest in T_1 group mice but the differences were not statistically significant among all groups of mice. The As contents increased in all treated group on day 63 compared to day 42 (Table 2).

DISCUSSION

In this study, the values of SGOT (Table 1) were increased significantly (P<0.01) in the blood samples of the treated groups of mice (T_1, T_2, T_3 and T_4) compared to control (T_0). Although this finding disagreed with the previous findings that SGOT was reduced by As alone (Mahaffey et al., 1981), it concurred with the findings of Yasmin et al. (2011) who indicated similar results. In Spirulina treated (T_2), Vitamin A treated (T_3) and Spirulina plus

Vitamin A treated (T_4) experimental arsenicosis groups, there were significantly decreased values of arsenic recorded ($P<0.01$) compared with the arsenic treated group of mice (T_1). Since arsenic toxicity can cause hepatic insufficiency and Spirulina and Vitamin A treatment improved the hepatic functions apart from decreasing the level of arsenic in blood, it can be concluded that Spirulina improve liver function by reducing hepatic damage due to heavy metal exposure and drug abuse (González et al., 1999). Although the levels of SGPT in serum differ significantly at day 21 and day 42 ($P<0.01$), it normalized in all groups and there was no significant difference by day 63 except for the arsenic group (Table 1). It is possible that individual treatment using Vitamin A or Spirulina or a combination of the two will not produce a significantly different result in long term arsenicosis. Interestingly, the level of SGPT did not change drastically in arsenic treated group (T_1) between days 42 and 63. It may be that onc a peak level of arsenicosis is reached, the SGPT level will adjust to it and maintain a peak value. Kaur et al. (2005) had earlier found that no change was observed in SGPT level associated with arsenicosis over a 90-day period. However, our result differed partly with this report as the 21 day collection of serum in our study revealed peak SGPT levels in arsenic treated group. Yasmin et al. (2011) had also recently reported a 16.67% increase in SGPT level of arsenic treated mice as compared to the control. Our findings also revealed that the TEC in the T_1 group was lower compared to control and other treatment groups. Whether this observation is due to relative or absolute anaemia cannot be established in this study. We however confirmed that treatment with vitamin A and Spirulina either alone or in combination improved the TEC, although the combination produced a better result. Chronic arsenic toxicity might cause decreased in TEC and anaemia. The effects observed in this study and outcomes of treatment using Spirulina plus vitamin A treatment had earlier been corroborated by Gupta et al. (2007); Breton et al. (2006) and Juruli and Katsitadze (2007). They reported decreasing RBC level with increased concentration of arsenic due to arsenic metabolism and its methylating activity. There was no significant difference in TLC and Hb values observed among all the groups during the entire study period but slight increase in TLC and Hb values (Table 2) in Mouse of all other treated groups (T_2, T_3 and T_4) compared to arsenic treated group (T_1) was observed. The result is similar to the findings of Yasmin et al. (2011); but contradicts that of Rousselot et al. (2004) where they found decreased WBC level and constant Hb level when mice were given higher dose of arsenic and that might be due to apoptotic effect of arsenic on plasma cells. It is possible that since arsenicosis is not an infectious condition, there was no mobilization of the physiologic system to increase the production of white blood cells or lymphocytes. Whether Spirulina and vitamin A had an influence on the values of TLC and Hb against arsenic toxicity in mice cannot be established in this study. A more carefully planned research targeting this objective is required to be undertaken.

CONCLUSION

Results illustrate that synergistic use of Spirulina and Vitamin A may be useful for the treatment of persistent arsenicosis in mice. The present study is a preface work on the potency of combined Spirulina and Vitamin A in arsenicosis in Bangladesh. Still, the result of this research work will positively facilitate the prospect researchers to afford direction in carrying out further detail study.

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